

We claim:

1. A method for identifying a unique nucleic acid capable of inducing lipid droplet formation in a cell, wherein said unique nucleic acid is present in a library, said method comprising:

- 5 (a) providing a library of a multitude of unique expressible nucleic acids, said library including a multiplicity of compartments, each of said compartments consisting essentially of one or more adenoviral vector comprising at least one unique nucleic acid of said library in an aqueous medium, wherein said adenoviral vector is capable of introducing said nucleic acid into a host cell, is capable of expressing the
- 10 product of said nucleic acid in said host cell, and is deleted in a portion of the adenoviral genome necessary for replication thereof in said host cell;
- (b) transducing a multiplicity of host cells with at least one adenoviral vector comprising at least one unique nucleic acid from said library;
- (c) incubating said host cells to allow expression of the product of said
- 15 nucleic acid; and
- (d) determining if a lipid droplet is formed in said cell.

2. The method of claim 1 wherein step (d) comprises observing said host cell to identify lipid droplet formation in said host cell relative to a host cell that has

20 not been transduced with an adenoviral vector comprising said nucleic acid.

3. The method of claim 1, wherein the function of the expression product of all of said unique expressible nucleic acids in said library is unknown at the time said library is first made.

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4. The method of claim 1, wherein none of said compartments contain any adenoviral vector capable of replication except in a packaging cell containing said deleted portion of said adenoviral genome.

30 5. The method of claim 1, wherein said host cell is a eukaryotic cell.

6. The method of claim 1, wherein at least one compartment comprises at least two adenoviral vectors.

5 7. The method of claim 1, wherein each of said compartments consists essentially of one said adenoviral vector.

8 The method of claim 4, wherein each of said compartments contains from about 0.01×10^{10} to about 10×10^{10} pfu of said adenoviral vector per ml of
10 aqueous medium.

9. The method of claim 8, wherein each of said compartments further contains the cellular debris from packaging cell lysate.

15 10. The method of claim 4, wherein said adenoviral vector is a minimal vector.

11. The method of claim 10, wherein said minimal vector comprises an adenovirus encapsidation signal or a functional part, derivative and/or analogue thereof, and at least one copy of at least a functional part or a derivative of an adenoviral ITR.
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12. The method of claim 4, wherein said adenoviral vector comprises adenoviral genomic sequence deleted for sequence encoding the E1-region proteins.
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13. The method of claim 11 wherein said minimal vector further comprises an adeno-associated virus terminal repeat or a functional part, derivative and/or analogue thereof.

14. The method of claim 12, wherein said adenoviral vector is further deleted for sequence encoding the E2A-region proteins, or the E2B region proteins or the complete E2 region proteins.

5 15. The method of claim 1, wherein said adenoviral vector further comprises adenovirus genomic sequence encoding adenoviral fiber proteins from at least two serotypes of adenovirus.

10 16. The method according to claim 1, wherein said multiplicity of cells is divided over a multiplicity of compartments, each said compartment comprising at least one vector.

15 17. The method according to claim 1, further comprising selecting at least one vector comprising a unique nucleic acid capable of inducing lipid droplet formation in a cell.

18. The method according to claim 1, wherein at least one of said performed steps is automated.

20 19. A method for obtaining an expressible nucleic acid capable of inducing lipid droplet formation when expressed in a cell, said method comprising:

(a) performing the method of claim 1;

determining which compartment in said library contains an adenoviral vector comprising a unique nucleic acid capable of inducing lipid droplet formation; and

25 obtaining said vector from said compartment.

20. The method of claim 1 wherein said host cell is a pre-adipocyte.

21. The method of claim 1 wherein said host cell is selected from the group consisting of pre-adipocytes, mesenchymal stem cells and progenitor cells.

22. The method of claim 1 wherein said lipid droplets are detected
5 microscopically.

23. The method of claim 22 wherein said microscopy is white light phase contrast microscopy.

10 24. The assay of claim 22 wherein said microscopy is fluorescence microscopy.

25. The method of claim 1 further comprising transducing the host cells in step (b) with an adenovirus encoding the receptor for adenovirus subtype 5(hCAR)
15 wherein said receptor is expressed in said host cell.

26. A method for determining whether the expression product of a nucleic acid, capable of inducing lipid droplet formation in a cell transfected with said nucleic acid, is secreted by said cell, comprising:

20 (a) infecting producer cells in a medium with an adenoviral vector comprising a unique nucleic acid capable of inducing lipid droplet formation;

(b) combining said medium with test cells that have not been infected with said vector; and

(c) determining if lipid droplets are formed in said test cells.

25 27. The method of claim 26 wherein said test cells are primary human pre-adipocytes.

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28. The method of claim 9, wherein the contents of each said compartment is capable of transfecting said host cell and expressing the product of each said unique nucleic acid in said host cell.

5 29. The method of claim 28, wherein each said compartment is capable of providing from about 10 to about 20 aliquots of said adenoviral vector.

30. The method of claim 11, wherein said minimal vector comprises a regulatable promoter operably linked to said unique nucleic acid.

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31. The method of claim 12, wherein said adenoviral vector comprises a regulatable promoter operably linked to said unique nucleic acid.

32. The method of claim 12, wherein said adenoviral vector is further
15 deleted for the adenoviral E3-region or a functional part thereof.

33. The method of claim 14, wherein said adenoviral vector is further deleted for the adenoviral E3-region or a functional part thereof.

20 34. The method of claim 32, wherein said adenoviral vector is further deleted for the adenoviral E4-region or a functional part thereof.

35. The method of claim 33, wherein said adenoviral vector is further deleted for the adenoviral E4-region or a functional part thereof.

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36. The method of claim 30, wherein said promoter is repressed by an adenoviral E1 gene product.

37. The method of claim 31, wherein said promoter is repressed by an adenoviral E1 gene product.

38. The method of claim 36, wherein said promoter is an AP1 dependent promoter.

39. The method of claim 37, wherein said promoter is an AP1 dependent promoter.

40. A method according to claim 1, wherein said adenoviral vector is packaged into an adenoviral capsid.

41. The method of claim 1 wherein said unique expressible nucleic acid is derived from the group consisting of mammals, fish, nematodes, insects, yeasts, fungi, bacteria and plants.

42. The method of claim 41 wherein said library of unique nucleic acid is derived from human placenta mRNA.

43. The method of claim 41 wherein said library of unique nucleic acid is derived from zebrafish mRNAs.

44. A method for identifying a unique nucleic acid capable of inducing lipid droplet formation in a cell, wherein said unique nucleic acid is present in a library, said method comprising:

(a) growing a plurality of cell cultures containing at least one cell, said one cell expressing adenoviral sequence consisting essentially of E1-region sequences and expressing one or more functional gene products encoded by at least one adenoviral region selected from an E2A region and an E4 region; and

(b) transfecting, under conditions whereby said recombinant adenovirus vector library is produced, said at least one cell in each of said plurality of cell cultures with

i) an adapter plasmid comprising adenoviral sequence coding, in operable configuration, for a functional Inverted Terminal Repeat, a functional encapsidation signal, and sequences sufficient to allow for homologous recombination with a first recombinant nucleic acid, and not coding for E1 region sequences which overlap with E1 region sequences in said at least one cell, for E1 region sequences which overlap with E1 region sequences in a first recombinant nucleic acid, for E2B region sequences other than essential E2B sequences, for E2A region sequences, for E3 region sequences and for E4 region sequences, and further comprises a unique nucleic acid sequence and promoter operatively linked to said unique nucleic acid sequence; and

ii) a first recombinant nucleic acid comprising adenoviral sequence coding, in operable configuration, for a functional adenoviral Inverted Terminal Repeat and for sequences sufficient for replication in said at least one cell, but not comprising adenoviral E1 region sequences which overlap with E1 sequences in said at least one cell, and not comprising E2A region sequences or E4 region sequences expressed in said plurality of cells which would otherwise lead to production of replication competent adenovirus wherein said first recombinant nucleic acid has sufficient overlap with said adapter plasmid to provide for homologous recombination resulting in production of recombinant adenoviral vectors in said at least one cell;

(c) incubating said plurality of cells under conditions which result in the lysis of said plurality of cells facilitating the release of said recombinant adenoviral vectors containing said unique nucleic acid; and

(d) transferring an aliquot of said adenoviral vectors into a corresponding plurality of host cell cultures consisting of cells in which said vectors do not replicate, but in which said nucleic acids are expressible;

(e) incubating said host cells to allow expression of the product of said nucleic acid; and

(f) observing said host cell for the presence of a lipid droplet

45. A method according to claim 44, wherein said lipid droplets are detected microscopically.

46. A method for identifying a drug candidate compound useful in the treatment of obesity, said method comprising:

(a) contacting one or more test compound with a polynucleotide comprising a sequence of SEQ ID NOS: 14, 16, 17 or 18, or the corresponding antisense sequence thereof,

(b) determining the binding affinity of said one or more test compound to said polynucleotide,

(c) contacting a first subpopulation of host cells transfected with said polynucleotide with one or more of said test compound that exhibits binding affinity for said polynucleotide, and

(d) identifying, from said one or more test compounds, a candidate compound that inhibits the formation of lipid droplets in said first subpopulation of host cells relative to a second subpopulation of said transfected host cells that have not been contacted with said candidate compound.

47. A method according to claim 46 wherein said test compound comprises a polynucleotide or a polypeptide.

48. A method for identifying a drug candidate compound useful in the treatment of obesity, said method comprising:

(a) contacting one or more test compound with a polypeptide expression product encoded by the polynucleotide comprising a sequence of SEQ ID NOS: 14 or 16,

(b) determining the binding affinity of said test compound to said polypeptide,

(c) contacting a first subpopulation of host cells transfected with a polynucleotide expression vector coding for said polypeptide with one or more of said test compound that exhibits binding affinity for said polypeptide, and

(d) identifying, from said one or more test compounds, a candidate compound that inhibits the formation of lipid droplets in said first subpopulation of host cells relative to a second subpopulation of said transfected host cells that have not been contacted with said candidate compound.

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49. A method according to claim 48 wherein said test compound comprises a polypeptide comprising the sequence of SEQ ID NO:15.

50. A method for identifying a drug candidate compound useful in the
10 treatment of obesity comprising:

(a) contacting one or more test compounds with a corresponding number of one or more first subpopulations of host cell transfected with an expression vector encoding a polynucleotide comprising a sequence of SEQ ID NOS: 14, 16, 17 or 18.

15 51. A method according to claim 50 further comprising

(b) selecting, from said one or more test compounds, a candidate compound that inhibits the formation of lipid droplets in said first subpopulation of host cell relative to a second subpopulation of said transfected host cell that have not been contacted with any test compound.

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52. A method according to claim 50 comprising

(b) selecting a candidate compound that results in an decrease in the expression of mRNA encoded by a polynucleotide comprising a sequence of SEQ ID NOS: 14, 16, 17 or 18 in said first subpopulation of host cell relative to the expression
25 of said mRNA in a second subpopulation of transfected host cells that has not been contacted with any test compound.

53. A method for identifying a drug candidate compound useful in the treatment of a disease state wherein said disease state is selected from the group

consisting of type II diabetes, hyperglycemia, impaired glucose tolerance, metabolic syndrome, syndrome X, dyslipidemia and insulin resistance, said method comprising:

(a) contacting a test compound with an expression product of the polynucleotide comprising a sequence of SEQ ID NOS: 14 or 16 or the corresponding antisense sequence thereof, and

(b) determining the binding affinity of said test compound to said expression product.

54. A method according to claim 53 comprising:

(c) contacting said test compound that exhibits binding affinity to said expression product with a first subpopulation of host cell, and

(d) selecting as a candidate compound, said test compound that causes an increase in the expression of mRNA encoded by a polynucleotide comprising a sequence of SEQ ID NOS: 14, 16, 17 or 18 in said first subpopulation of host cells relative to the expression of mRNA in a second subpopulation of host cell that has not been contacted with said compound.

55. A method according to claim 53 further comprising:

(c) contacting said test compound that exhibits binding affinity for said expression product with a first subpopulation of host cells transfected with an expression vector encoding said polypeptide, and

(d) selecting as a candidate compound, said compound that exhibits said binding affinity and that enhances the formation of lipid droplets in said first subpopulation of host cells relative to a second subpopulation of said transfected host cells that have not been contacted with said binding affinity compound.

56. A method for identifying a drug candidate compound useful in the treatment of a disease state wherein said disease state is selected from the group consisting of type II diabetes, hyperglycemia, impaired glucose tolerance, metabolic syndrome, syndrome X, dyslipidemia and insulin resistance, said method comprising:

(a) contacting one or more test compounds with a corresponding number of one or more first subpopulations of host cell transfected with an expression vector encoding a polynucleotide comprising a sequence of SEQ ID NOS: 14, 16, 17 or 18.

5 57. A method according to 56 further comprising

(b) selecting, from said one or more test compounds, a candidate compound that enhances the formation of lipid droplets in said first subpopulation of host cell relative to a second subpopulation of said host cell that have not been contacted with any test compound.

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58. A method according to claim 56 comprising

(b) selecting a candidate compound that results in an increase in the expression of mRNA encoded by a polynucleotide comprising a sequence of SEQ ID NOS: 14, 16, 17 or 18 in said first subpopulation of cell relative to the expression of said mRNA in a second subpopulation of host cells that has not been contacted with said any test compound.

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59. An isolated antibody that specifically binds to the polypeptide expression product encoded by the polynucleotide comprising the sequence of SEQ ID NO:14.

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60. A pharmaceutical composition comprising a polypeptide expression product encoded by the polynucleotide comprising the sequence of SEQ ID NO:14 and a pharmaceutically acceptable carrier.

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61. A pharmaceutical composition comprising a polynucleotide sequence of SEQ ID NOS: 14, 16, 17 or 18 and a pharmaceutically acceptable carrier.

62. A pharmaceutical composition comprising a polynucleotide complementary to the sequence of SEQ ID NOS: 14, 16, 17 or 18 and a pharmaceutically acceptable carrier.

63. A method for treating a disease state selected from the group consisting of type II diabetes, hyperglycemia, impaired glucose tolerance, metabolic syndrome, syndrome X, dyslipidemia and insulin resistance, said method comprising administering to a patient in need of such treatment an effective adipogenesis amount of the composition of claim 60.

64. A method for treating a disease state selected from the group consisting of type II diabetes, hyperglycemia, impaired glucose tolerance, metabolic syndrome, syndrome X, dyslipidemia and insulin resistance, said method comprising administering to a patient in need of such treatment an effective adipogenesis amount of the composition of claim 61.

65. A method for treating obesity, said method comprising administering to a patient in need of such treatment an effective anti-obesity amount of the composition of claim 62.

66. An expression vector comprising a polynucleotide sequence of SEQ ID NOS: 14, 16, 17 or 18.

67. A method for treating a disease state selected from the group consisting of type II diabetes, hyperglycemia, impaired glucose tolerance, metabolic syndrome, syndrome X, dyslipidemia and insulin resistance, said method comprising administering to host cells of a patient in need of such treatment an effective adipogenesis amount of the expression vector of claim 66.

68. An expression vector comprising a polynucleotide complementary to a polynucleotide sequence of SEQ ID NOS: 14, 16, 17, or 18 wherein said vector is capable of expressing said polynucleotide.

5 69. A method of treating obesity comprising administering to host cells of a patient in need of such treatment the expression vector of claim 68.

70. A method according to claim 50 wherein said cells are primary cells.

10 71. A method according to claim 70, wherein said primary cells are selected from the group consisting of adipocytes, pre-adipocytes, mesenchymal stem cells, and progenitor cells.

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